Draft Responses to RDX Charge Questions

Revised Draft Responses to Charge Questions based on Discussion on December 14, 2016 Public Session. Do not cite or quote.

Charge Question #1: Literature search/study selection and evaluation

The section on Literature Search Strategy, Study Selection, and Evaluation describes the process for identifying and selecting pertinent studies.

Please comment on whether the literature search strategy, study selection considerations including exclusion criteria, and study evaluation considerations, are appropriate and clearly described.

Please identify additional peer-reviewed studies that the assessment should consider.

Charge Question 1 Response

- The literature searching strategy was clearly described.
- The comprehensiveness of the literature search strategy was good, with exceptions as noted below:
  - MNX, DNX, TNX should have been included
  - MEDINA & related oxidative transformation products should have been included
  - Evaluation of differential male and female sensitivities to GABA dysfunction was lacking
  - Description of the role of GABA in brain development should be included
- Inclusion/Exclusion criteria were well described and for the most part appropriate, except that:
  - Exclusion of non-mammalian studies may not be appropriate given current use of zebrafish and other non-mammalian models for Adverse Outcome Pathway determination
  - The exclusion of data for reasons of purity are not well supported, and exposure to impurities occurs in real life. In some cases water was the impurity, which is needed to minimize ignition hazard.

Added References

- Data describing toxicity of MNX (CID: 535289) and TNX (CID: 26368) should be included. These reductive transformation products are:
  1) present in ground waters near munitions and training facilities
  2) produced in the GI tract of mammals
  3) present in the blood and target tissues of dosed mammals
  4) structurally similar to di-N-nitroso-piperazine (CID: 8490; CASRN: 140-79-4), a known carcinogen
- The Committee assembled 15 candidate references that address the toxicity of reductive transformation products and were conducted in species that may inform the current RDX assessment.
- We have identified 6 references that may be used to start the discussion of the role of GABAergic systems during development and the potential for RDX developmental neurotoxicity.

Charge Question #2: Toxicokinetic Modeling

2a. PBPK model scientifically supported

- The PBPK model used in the RDX IRIS assessment is a reasonable model for application in this assessment.
  - The model and choices in its development are well documented and supported by the available scientific information, which is adequate but limited.
  - EPA improved the published models.
  - The uncertainties in the model are well described.
- Limitations of the available data include:
  - Lack of plasma protein binding and tissue concentration data;
    - Fat and muscle partition coefficients may be poorly estimated leading to mis-estimation of volume of distribution and clearance
  - In vitro data for use in predicting clearance in the different species
  - Limited or lacking data on metabolites, including reductive metabolites. EPA could confirm that no additional data (e.g., references provided) are informative, probably qualitatively.
  - Variations in RDX formulations (e.g., particle size) create uncertainties in the characterization of absorption (rate and possibly extent) as noted in the document. Plasma concentrations following oral dosing do not include enough early time points to properly estimate absorption parameters, so as noted Cmax is particularly uncertain.

2b. Dose Metric Selection

- Use of plasma AUC for parent compound is preferred over Cmax
- Blood RDX AUC is the preferred and reasonable dosimeter for neurotoxicity due to:
  - data showing proportional changes in blood and brain RDX concentrations over time following oral dosing;
  - concordance between RDX levels and symptomatology over an extended period of hours following exposure, as opposed to the Cmax at a single point in time.
- For other rat toxicity endpoints, the rationale for selection of AUC needs to be stated.
- Uncertainty in the role of parent compound versus metabolites should be noted for these endpoints.

2c Human Variability Uncertainty Factor

- Given the limitations of the available data, it would not be reasonable to assess human variability using a PBPK model, and therefore, use of the default UF_H is supported.
- Sensitivity analyses showed that model output was substantially impacted by bioavailability and the metabolic rate constant. Metabolic clearance is an input factor for which there may be substantial inter-subject variability.
- For future analyses, data on $GABA_A$ binding or response variability could inform identification of sensitive populations or help to characterize TD variability.
- Potential metabolite toxicity, and potential species differences in metabolites, may contribute to uncertainty factor.

Charge Question #3a(i) Nervous System Hazards

3a(i) The draft assessment concludes that nervous system toxicity is a human hazard of RDX exposure. **Comment on whether the available human, animal, and mechanistic studies support this conclusion?**

Response: **Yes**

- Human studies
  - Several case reports of convulsions and behavior changes without exposure measures
  - Occupational study (Ma and Li 1993) show neurobehavioral cognitive effects but lack exposure measures for the unexposed group, or control for confounders
  - Rationale unknown for subgrouping
- Animal studies
  - Rodent studies
    - Subchronic and chronic
    - Gavage and dietary
    - Convulsions
    - Seizures measured visually observing behavior (incidental)
    - Other signs of behavioral toxicity (aggression, incidental)
    - Dose better predictor than duration
    - May sensitize to lower doses on subsequent exposure (kindling)
- Mechanism studies: RDX blocks GABA-A receptors; relatively low potency (mid μM), but long-lasting

3a(i) Cont'd. Are all hazards to the nervous system adequately assessed?

Response: **No**

- Lack of full spectrum of neurotoxicity endpoints
  - Subchronic sensitization; neuroinflammation
  - EEG seizures; seizure threshold; hyper-reactivity (behavioral ethogram)

3a (i) Cont'd. Is there an appropriate endpoint to address the spectrum of effect?

Response: **Yes**

- Convulsions; tremors; aggression

3a(ii): **Nervous system-specific toxicity values**

Please Comment on whether the selection of studies reporting nervous system effects is scientifically supported and clearly described.

Response: **YES**

- Limited, but scientifically acceptable studies
- Database is limited on subtle neurological outcomes (database uncertainty factor?)

Considering the difference in toxicokinetics between gavage and dietary administration , is it appropriate to consider the Crouse et al. (2006) study, which used gavage administration?

Response: **YES**

- Gavage study can be protective of dietary exposure, but unlikely pulmonary exposure.
- Crouse study has the most dose points and longer in duration.

3a(ii) Cont'd Nervous system-specific toxicity values

Is the characterization of convulsions as a severe endpoint, and the potential relationship to mortality, appropriately described?

Response: YES

- There are some uncertainties regarding convulsions—lethality relationship.
- Mortality can arise from non-neurotoxicity factors

Charge Question 3a(iii) Points of departure for nervous system endpoints.

1. ***Is selection of convulsions as the endpoint to represent this hazard scientifically supported and clearly described?***

Response: **YES**.

- Evidence from other seizurogenic compounds with similar modes of action suggest more subtle cognitive and behavioral neurological effects exist for RDX. However, no such data exist for RDX. LOELs for triggering abnormal electrographic patterns and for convulsions are within a factor of 2-3 fold dose range.
  - can be addressed with UFs

3a(iii) Cont'd

- ***Are the calculations of PODs for these studies scientifically supported and clearly described?***

Response: Given EPA's choice of critical study and BMR?, the POD for convulsions was calculated correctly. **However, see discussion below regarding the use of a BMR of 1% and the response to question 4a regarding the choice of the critical study.**

3a(iii) Cont'd

Is the calculation of the HEDs for these studies scientifically supported and clearly described?

Response: YES

EPA used AUC. Using a PBPK model, and given binding of parent compound to the GABA receptor, AUC is a reasonable choice. Use of Cmax is more limiting due to absorption parameters and their variability with the formulation of RDX

3a(iii) Cont'd

Does the severity of convulsions warrant the use of a benchmark response level of 1% extra risk?

Response: **NO**

- Issues
  - BMR of 10 % is the default for quantal data- but not necessary or required.
    - Typical justification for using lower BMR is biological considerations.
    - 1% BMR recommended for Epi data
  - **However**, also necessary to weigh the distribution of the dose-response data (per EPA Benchmark Dose Technical Guidance)
  - Uncertainty increases with extrapolation of estimates at BMRs below observable range of response data.
  - Proximity of dose response for convulsions to dose-response for lethality is a valid source of uncertainty, but benchmark dose modeling should objectively describe the dose-response. Uncertainty should be addressed through uncertainty factors.

Existing Data
LOAELs for convulsions (frank effect)
Among these data lowest response is 4%, with Crouse (EPA's critical study) at 15% sensitivity

Study	n/dose group	LOAEL	Response at LOAEL
Crouse	10 rats/sex/dose group	8 mg/kg/d	15%
Cholakis	24-25 pregnant F/dose group	2 mg/kg/d	4%
Martin & Hart (monkeys)	3/sex/dose group	10 mg/kg/d	83%

Recommendation

- Recommend Use of BMR of 5% for Crouse et al.
  - Departure from 10% justified as frank effect in animals More in line with assay sensitivity (15% for Crouse et al.) compared with 1%
  - Estimate is not far outside the observable range of effective doses (≥ LOAEL) that uncertainty in dose-response extrapolation becomes a major factor

  - Crouse (LOAEL 8):
    - $BMD_{1\%}$ = 3.02 (0.569)
    - $BMD_{5\%}$ = 5.19 (2.66)
    - $BMD_{10\%}$ = 6.60 (4.59)

  - Cholakis (LOAEL 2):
    - $BMD_{1\%}$ = 0.179 (0.123)
    - $BMD_{5\%}$ = 0.915 (0.628)
    - $BMD_{10\%}$ = 1.88 (1.29)

3a(iii) Cont'd

Is calculation of the lower bound on the benchmark dose (BMDL) for convulsions appropriate and consistent with the EPA's Benchmark Dose Guidance?

Response: **YES**

- Model selection was appropriate

3.a.iv. Uncertainty factors for nervous system endpoints (Section 2.1.3). Is the application of uncertainty factors to these PODs scientifically supported and clearly described? The subchronic and database uncertainty factors incorporate multiple considerations; please comment specifically on the scientific rationale for the application of a subchronic uncertainty factor of 1 and a database uncertainty factor of 3

3.a.iv.UF for nervous system toxicity using Crouse et al., 2006

- UF_A of 3 to account for uncertainty in inter-species differences in toxicodynamics (TD) and residual toxicokinetics (TK) when using a PBPK model for extrapolation from animal to human is standard risk assessment practice and we concur with this decision.
- UF_S of 1; the committee identified some concern for sensitization to additional doses from longer term exposure, but if kindling would occur it would be expected to occur within timeframe of studies (2 weeks-90 days) and no evidence of increasing response over time observed in the available data.
- UF_L – not needed as had BMDL
- UF_H – standard 10 fold for TK and TD differences among humans

3.a.iv.UF for nervous system toxicity using Crouse et al., 2006

- UF_D of 3 applied by USEPA
- No developmental neurotoxicity study despite transplacental and lactational transfer, and other data streams raising concern
- Severe effect is the basis of RfD; convulsions and mortality occur at similar doses (BMDL01 similar to LD01s)
  - No studies evaluating incidence of less severe neurotoxicity
  - Convulsions seen at lower doses in Cholakis and death at lower doses in Angerhofer.

- Recommendation: EPA should consider UF_D of 10 to account for data gaps for developmental neurotoxicity, lack of incidence data for less severe effects, proximity of BMDL01 to LD01.

3a(v): Nervous system-specific reference dose. Is the organ/system- specific reference dose derived for nervous system effects scientifically supported and clearly characterized?

Response: **No**

- RfD did not capture all of the potential adverse outcomes.
- The Committee considered NOAEL from Cholakis et al 1980 in combination with Crouse et al. 2006 dose-response data. Discussed in 3a(iii) & 3a(iv)
- The severity of outcome does not accurately reflect the 3-fold uncertainty factors

RfD: 3 μg/kg/day ⟹ RfD: 1 μg/kg/day

3.b.i. ***Kidney and other urogenital system hazard*** *(Sections 1.2.2, 1.3.1). The draft assessment concludes that kidney and other urogenital system toxicity is a potential human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support this conclusion. Are all hazards to kidney and urogenital system adequately assessed? Is the selection of suppurative prostatitis as the endpoint to represent this hazard scientifically supported and clearly described?*

Do available human, animal, and mechanistic studies support this conclusion:

- Yes, but the conclusion that kidney and other urogenital system toxicity is a potential human hazard of RDX exposure is primarily supported by animal data.
- Available human studies are sparse but consistent with the conclusion that the kidney may be a target of RDX in humans. There are no reports of prostatic effects in humans.
- There are no pertinent mechanistic data.

Are all hazards to kidney and urogenital system adequately assessed:

- Yes, all hazards to kidney and urogenital system are adequately assessed and described, except for the description of inflammatory changes in the rat prostate that include not only the suppurative inflammation described in the draft assessment, but also chronic inflammation.

3.b.i. - continued: Is the selection of suppurative prostatitis as the endpoint to represent this hazard scientifically supported and clearly described?

- The selection of suppurative prostatitis as the endpoint ("surrogate marker") to represent this hazard is clearly described, but not scientifically supported because of various uncertainties:
  - There is no known biological basis for using this lesion as a surrogate marker for renal and other GU effects.
  - Uncertainty of whether suppurative prostatitis is associated with the renal toxic effects which were found only at the highest dose.
  - Uncertainties about the diagnosis of suppurative inflammation:
    - Suppurative and chronic inflammation are part of a continuum and diagnostic criteria may have varied over time and among pathologists.
    - Lack of details about the histopathology methods in the Levine *et al*. (1983) report given the known large variation in inflammation across prostate lobes based on NTP data of aged F344 rats.
    - Absence of peer review or pathology working group review of the Levine *et al*. (1983) data.
    - Combining all types of inflammation in the Levine *et al*. (1983) study yields similar incidences in all groups, except the highest dose group, and these incidences are consistent with NTP data of aged F344 rats.
    - Potential effects of the high prevalence of fighting in highest dose rats and consequent individual housing of all males in the Levine *et al*. (1983) study.

3.b.i. - continued:

- Recommendations:
  - Do not use the suppurative prostatitis as a "surrogate marker" of renal and overall GU effects. Instead, consider these as separate effects (see also 3.b.v).
  - Improve the description and analysis of prostatitis to include both chronic and suppurative inflammation.
  - Improve the description of the various uncertainties regarding the Levine *et al*. (1983) rat study.

3.b.ii. ***Kidney and other urogenital system-specific toxicity values*** *(Section 2.1.1). Is the selection of the Levine et al. (1983) study that describes kidney and other urogenital system effects scientifically supported and clearly described?*

- The selection of the Levine *et al*. (1983) study which found kidney and other urogenital system effects is clearly described, but not fully supported scientifically.
- While the renal effects found in male rats of the highest dose group by Levine *et al*. (1983) were obvious, associated mortality, and treatment-related, the effects on the bladder and particularly the prostate were milder and less straightforward (see also 3.b.v).
- The study referenced above was not the only animal study that found effects on kidney. Renal medullary mineralization was found in male and female Cynomolgus monkeys and cortical tubular nephrosis was found in male mice (only at a very high RDX dose); both studies were of 13 weeks duration and the renal effects were minimal to moderate in severity and not or only marginally significant.
- The marked sex difference in renal toxicity due to RDX found for rats by Levine *et al*. (1983) was not discussed in the draft assessment. There is precedent for toxic chemicals causing renal papillary necrosis selectively in one sex.
- <u>Recommendation</u>:
  - Briefly discuss the marked sex difference in the renal toxicity due to RDX effects in rats.

Charge Question 3b(iii). Points of departure for kidney and other urogenital system endpoints.

1. Is the calculation of a POD for this study scientifically supported and clearly described? YES
2. Is the calculation of the HED for this study scientifically supported and clearly described? YES

<u>** All contingent on using suppurative prostatitis as an endpoint **</u>

- *A strong recommendation to the EPA is to treat suppurative prostatitis as a stand-alone endpoint, separate from kidney and other urogenital system endpoints.*

- Ten models were fit to available animal data, with all models having acceptable goodness of fit and very similar AIC estimates.
- $BMD_{10\%}$ estimates ranged from 1.67 to 10.8 across the ten models, with $BMDL_{10\%}$ ranging from 0.469 to 8.58.
- As can be seen in Figure D-7, the selected log-probit model fits the data well, with an estimated $BMD_{10\%}$ of 1.67, which is within the range of study doses, thus obviating any issues of inappropriate extrapolation.

- The scientific data are variable with respect to toxicity involving the kidney and urogenital effects and the effect of metabolites is unknown. However, the HED appears appropriate for kidney/urogenital effects based on scientific literature.
- The alternative, allometric scaling from administered dose to rats, introduces its own uncertainties. More would be helpful here.

Charge Question 3b(iv) Uncertainty factors for kidney and other urogenital system endpoints - Is the application of uncertainty factors to the POD scientifically supported and clearly described?

Charge Question 3b(iv). Uncertainty Factors using Levine et al., for urogenital system hazard

- UF_A of 3 to account for TD and residual TK when using TK extrapolation from animal to human is standard risk assessment practice and we concur with this decision.
- UF_L – not needed as had BMDL
- UF_S of 1 standard with 2 year study.
- UF_H 10 fold for TK and TD differences among humans is standard practice.

Charge Question 3b(iv). Uncertainty Factors using Levine et al., for urogenital system hazard

- UF_D of 3 applied by USEPA to account for inadequacies in the database for characterizing the neurotoxicity hazard.
- The recommendation for UF_D of 10 should be applied to the overall RfD.
- For endpoint-specific RfD, a different UF_D may be warranted.
- Unclear from assessment how organ-specific RfDs would be used, and recommend EPA develop and document methods for their derivation and use.

3.b.v. ***Kidney and other urogenital system-specific reference dose*** *(Section 2.1.4). Is the organ/system-specific reference dose derived for kidney and other urogenital system effects scientifically supported and clearly characterized?*

- **No**. The selection of suppurative inflammation of the prostate observed in the Levine *et al*. (1983) study as "surrogate marker" of the observed renal and GU effects is not justified for derivation of a reference dose (RfD) (see 3.b.i).
- Separate RfDs could be considered for renal papillary necrosis & associated inflammation, suppurative prostatitis, and other, milder renal effects (tubular nephrosis and mineralization) found in subchronic studies in mice and monkeys.
- Some of the renal and bladder lesions that were treatment-related at the highest doses tested in the various species also occurred in one or two animals in lower dose groups, sometimes with marked severity. These lesions are almost certainly "spontaneous" and not RDX treatment-related and should, therefore. not be used to derive an RfD.
- Recommendation:
  - Separate the renal and prostatic effects for the purpose of quantitative risk assessment. (The renal effects were clearly limited to male rats in the 40 mg/kg-day dose and high dose monkeys and mice, whereas the prostatic effects in male rats may also have been present at lower doses, but with a low level of confidence).

Charge Question 3c(i)

Developmental and Reproductive System Hazard

The draft assessment concludes that there is suggestive evidence of male reproductive effects associated with RDX exposure, based on evidence of testicular degeneration in male mice.

Response: **No** – Available animal data do not support the conclusion that there is suggestive evidence of male reproductive effects

Rationale: Summary of Results of 7 Studies of Male Reproductive Toxicity of RDX

- 5 rat and 2 mouse studies
- Histologic changes in 2 chronic studies – at specific time points but not at other time points.
- 5 had no histopathogical changes.
- All 13-week subchronic studies did not show any testicular toxicity.

Charge Question 3c(i) Developmental and Reproductive System Hazard (Cont'd)

The draft assessment did not draw any conclusions as to whether developmental effects are a human hazard of RDX.

Response: **No** – We concluded based on the data reviewed, that there is available evidence in animals indicating that RDX exposure does not represent a teratogenic hazard to humans.

Additionally, we agree that conclusions cannot be drawn regarding others forms of developmental toxicity, which occurred only at maternally toxic dose levels

Charge Question 3c(i)
Developmental and Reproductive System Hazard (Cont'd)

Are other hazards to human reproductive and developmental outcome adequately addressed?

Response: **No** – A mechanistic concern (inhibition of GABAergic neurons) for developmental neurotoxicity exists

- The report should consider noting the lack of an assessment for functional or neurobehavioral deficits in F1 generation animals. With detectable levels of RDX in the blood, brain and milk, at a dose that induced convulsions in adult animals.

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Charge Question 3c(ii) Developmental and Reproductive System Hazard

Is the selection of the Lish et al.(1984) study that describes male reproductive system effects scientifically supported and clearly described?

Response: **No** – Consideration of all evidence presented does not support the selection of Lish 1984 for male reproductive effects. (See chart on next slide)

Study	Model	Route	Significant Effect	**Doses** mg/k/d **Time** months	Caveats	Negative Result
Lish	Mouse	Diet	10% increase in incidence of testicular degeneration (sig?)	35 & 108 24 mo.	Mortality (>14%)	No histological change at 6 or 12 mo. No decrease in testis weight
Cholakis	Mouse	Diet	None	40, 80, 160, 320 3 mo.		No histological changes No decrease in testis weight
Levine ’83	Rat	Diet	40% increase in incidence of germ cell degeneration 14% decrease in testis weight	40 12 mo.	Mortality 27%	No effects at 8 mg/kg-day No effects at 6 months with 40 mg/kg-day Germ cell degeneration not seen at 40 mg/kg-day at 24 mo.
Hart	Rat	Diet	None	10 24 mo.		No histological changes No decrease in testis weight
Cholakis	Rat	Diet	18% reduction in proportion of females impregnated	50 3 mo	Mortality 14%; Possible behavioral effect	No histological changes or decreases in testis weight at 40 mg/kg-day
Levine ‘81	Rat	Diet	None	10, 30 & 100 3 mo.		No histological changes No decreases in testis weight
Crouse	Rat	Gavage	None	10, 12, 15 3 mo.		No histological changes No decreases in testis weight

Charge Question 3c (iii) Points of departure for reproductive system endpoints.

1. Is the calculation of a POD for this study scientifically supported and clearly described? YES
2. Is the calculation of the HED for this study scientifically supported and clearly described? YES

<u>** All contingent on using testicular degeneration as the endpoint **</u>

Charge Question 3c(iii) Cont'd

- Ten models were fit to available animal data, with all models having acceptable goodness of fit.
- $BMD_{10\%}$ estimates ranged from 56.0 to 97.1 across the ten models, with $BMDL_{10\%}$ ranging from 16.3 to 66.1.
- As can be seen in Figure D-6, the selected log-probit model fits the data well, with an estimated $BMD_{10\%}$ of 56 which is well within the range of study doses, so there is no issue of inappropriate extrapolation.

- Allometric scaling of mouse dose is the least uncertain of the options in this case given the limitations on data supporting the mouse parameterization of the PBPK model and uncertainty around active form of the compound.

Charge Question 3 c (iv). Uncertainty factors for reproductive system endpoints (Section 2.1.3). Is the application of uncertainty factors to the POD scientifically supported and clearly described?

Charge Question 3.c (iv) Uncertainty Factors using Lish et al, testicular toxicity as representing reproductive hazard

- UF_A of 3 to account for TD and residual TK when using TK extrapolation from animal to human is standard risk assessment practice and we concur
- UF_L – not needed as had BMDL
- UF_S of 1 standard with 2 year study.
- UF_H 10 fold for TK and TD differences among humans is standard practice.

Charge Question 3c(iv) Uncertainty Factors using Lish et al, testicular toxicity as representing reproductive hazard

- UF_D of 3 applied by USEPA.
- No developmental neurotoxicity study despite transplacental and lactational transfer and other data streams raising concern.
- The recommendation for UF_D of 10 be applied to the overall RfD.
- For endpoint-specific RfD, a different UF_D may be warranted.
- Unclear from assessment how organ-specific RfDs would be used, and recommend EPA develop and document methods for their derivation and use.

Charge Question 3c(v). Reproductive System-specific reference dose

Is the organ/system-specific reference dose derived for reproductive system effects scientifically supported and clearly characterized?

Response: **No** – Since the selection of Lish 1984 for representing the male reproductive effects was not supported, the reference dose calculated from it cannot be supported.

Charge Question 3d. Other Non-cancer Hazards

3d. The draft assessment did not draw any conclusions as to whether liver, ocular, musculoskeletal, cardiovascular, immune, or gastrointestinal effects are human hazards of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support this decision.. Are other non-cancer hazards adequately described?

- Recommendations:
  - For the "other non cancer hazards" mentioned in Sections 1.2.4, 1.2.6, and 1.3.1., provide a conclusion, i.e., a specific statement, regarding evidence of human hazard, rather than using the statement "no conclusions are drawn."
  - The evidence for these other effects was well described and summarized, except for:
    - neuroinflammatory changes that may influence immune system conclusions (as, e.g. in Dey et al., 2016; Trends in Pharmacological Sciences).
    - dose-related effects on body weights and/or body weight gains should be addressed.
  - Bring summaries for each of the listed endpoints from the Appendix into the Toxicological Review.

3e.i. Cancer hazard (Sections 1.2.5, 1.3.2). There are plausible scientific arguments for more than one hazard descriptor as discussed in Section 1.3.2. The draft assessment concludes that there is *suggestive evidence of carcinogenic potential* for RDX, and that this descriptor applies to all routes of human exposure. Please comment on whether the available human, animal, and mechanistic studies support these conclusions.

- The panel agrees that available human, animal, and mechanistic studies support the descriptor that there is "*suggestive evidence of carcinogenic potential* for RDX" and this descriptor applies to all routes of human exposure.
- Several limitations were identified in the available studies, namely the studies by Lish *et al.* (1984) and Levine *et al.* (1983)
  - A high mortality rate at the highest dose in both studies
    - In rats, the high mortality was likely due to renal disease
    - In mice, the high mortality was due to sub-acute toxicity
  - A low incidence of hepatocellular tumors in control female mice in the Lish et al. (1984) study, when compared to NTP controls (1.5 vs. 8.0%).
  - The lack of a pathology peer-review for neoplastic lesions in the rat study, for the liver tumors in male mice, and for the lung tumors in male and female mice
  - Lack of available data from the Lish *et al.* and Levine *et al.* studies to conduct mortality-based statistics
- The modes of action (MOA) of RDX (genotoxic versus non-genotoxic) are not well understood and mechanistic studies are inadequate.

3e. ii. Cancer-specific toxicity values (Section 2.3.1). As noted in EPA's 2005 Guidelines for Carcinogen Risk Assessment, "When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the nature of the data generally would not support one; however, when the evidence includes a well-conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities." Does the draft assessment adequately explain the rationale for quantitative analysis, considering the uncertainty in the data and the suggestive nature of the weight of evidence, and is the selection of the Lish et al. (1984) study for this purpose scientifically supported and clearly described?

- Panel finds the draft assessment adequately explains the rationale for quantitative analysis considering the uncertainty of the data and the suggestive nature of the weight of evidence.
- Panel finds that the selection of the Lish *et al.* (1984) study for this purpose is scientifically supported and clearly described.

Charge Question 3 e(iii). Points of departure for cancer endpoints

Are the calculations of PODs and oral slope factors scientifically supported and clearly described?

Response: **NO**.

The Panel finds the POD calculation approach is not clearly described.

There are concerns with the quality of the scientific support of the methodology used.

Charge Question 3e(iii). Concerns

- Paucity of data on mechanisms of action – no support for dose response model form.
  - Two mechanisms of action are proposed- genotoxicity and oxidative stress.
  - Published data are inadequate (insufficient) to conclude modes of genotoxic or non-genotoxic mechanisms of carcinogenesis
  - The Panel discussed needed future research to close this data gaps
  - Without a clear mode of action, the default approach to using linear low-dose extrapolation which was used in the RDX draft assessment is supported as recommended in EPA 2005 cancer guidelines.
- Low incidence of liver tumors (hepatocellular adenomas and carcinoma) in female mice and its impact on dose response modeling.
  - As indicated in Section 1.2.5, the 1.5% incidence of liver tumors in the control B6C3F1 mice was unusually low. This was reported by the study authors as significantly lower than those in historical controls, and is lower than the incidences seen in this strain by the NTP (mean 8%, range 0-20%).
  - This unusually low control incidence could significantly influence the estimate of the POD.

Charge Question 3e(iii). Concerns

- While the multistage model provides an acceptable fit to the data in the benchmark dose modeling, use of other models should be explored.
  - Rationale for restricting modeling to the multistage model is not compelling, and other models could provide a better fit of the data.
    - This may negate to some extent the influence of the high dose on the POD (see following point)
- Use of the multistage model in the MS-COMBO methodology is not well described and the situation necessitating the use of the multistage model and MS-COMBO is not clearly described.
    - Animal data on joint occurrence of tumors from pathology review was not available
    - The precise definition of "independence" as it relates to tumor types/locations in the MS-COMBO methodology should be clearly delineated and the evidence supporting the use of this assumption should be presented in that context.
    - Does MS-COMBO require adequate multistage model fit for each tumor?

Charge Question 3e(iii). Issues

- The Panel identified concerns with use of the highest dose in dose response modeling and its impact on the POD estimate?
  - Highest dose change at week 11 due to high mortality.
  - Exclusion of animals that died prior to week 11, resulted in a sample size decline in the highest treatment group (65 to 31 animals) and subsequent increase in uncertainty in response for this treatment group.
  - Previous risk assessment using this study excluded the high dose in deriving the cancer slope factor.

  - Use of models other than the multistage model for benchmark dose modeling may better accommodate the high dose and this may provide an alternative to its elimination from modeling.
  - While survival times of highest dose group is not significantly different from other dose groups, high mortality in the early weeks may mean that remaining survivors may have other differences that could result in higher resistance to cancer.
  - Excluding the highest dose group combined with the near linear estimated dose response curve form will significantly reduce the POD – resulting in an unrealistically high estimated cancer slope factor - Figure D-15).

Charge Question 3e(iii) Recommendations

- The report should discuss the independence assumption relative to the incidence of liver and lung cancers, using available data to support the validity of that assumption.
- The report should present the results of BMD modeling of each cancer separately, including not just the multi-stage model but the full suite of models used in non-cancer endpoints – justify adequacy of multi-stage model for MS-COMBO analysis.
- For liver cancer, the BMD modeling should explore the impact of low concurrent controls on model choice and POD estimate – sensitivity analysis.
- Describe the MS-COMBO methodology in more detail in the Appendix including labeling and describing better the program output.

Charge Question 4a. Oral reference dose for effects other than cancer

- *Is the selection of an overall oral reference dose of 3 × 10-3 mg/kg-day, based on nervous system effects as described in the Crouse et al. (2006) study, consideration of mortality as described in Section 2.1.6, and consideration of the organ/system-specific reference dose derived from the toxicity study by Cholakis et al. (1980) that is lower (by approximately fivefold) as described in Section 2.1.4, scientifically supported and clearly described ?*

Charge Question 4a

Response:

- Reasonably well described
- Scientific support for the proposed oral reference dose was weak
  - Does not take into account confirmed convulsion in exposed pregnant females at 2 mg/kg/day in the Cholakis et al. (1980).
    - `incomplete observation does not argue against Cholakis, but rather implies underestimation of risk.
    - LOAEL based on 1 animal, but frank effect

Charge Question 4a

- Tighter dose spacing and cleaner model fit from Crouse does not eliminate the lower NOAEL/LOAEL from Cholakis

Considerations for using Cholakis

- BMD analysis
  - But high dose in Cholakis includes non-convulsion effects (EPA)
  - Elimination of high dose leaves only one effect dose
    - This does not provide an appropriate basis for benchmark dose modeling
  - The panel rejected this option

- Combine dose-response data from Cholakis and Crouse
  - Different exposure durations
    - Cholakis – 13 days; Crouse – 13 weeks
  - Sex and pregnancy status differences
    - Crouse based on males + females
    - Cholakis based on pregnant animals only
    - Females
      - More GABA receptors than males
  - The panel rejected this option

Charge Question 4a

- Use NOAEL (0.2 mg/kg/d) from Cholakis
- (Panel's Recommendation)

  - With elimination of high dose in Cholakis (which includes non-convulsion effects) there is no basis for BMD anyway.
  - This option eliminates problem with choice of appropriate BMR from Crouse
  - Addresses lower NOAEL/LOAEL from Cholakis
  - Using same UFs as EPA, RfD
    - Cholakis = 1×10^{-3} mg/kg/d
    - Crouse = 3×10^{-3} for BMR = 1%, but
    - = 5×10^{-3} for BMR = 5%

Consideration of Mortality

- Mortality and convulsion are linked
- However, there is no evidence of (neurologic) mortality in the absence of convulsions
- Cholakis RfD is based on a NOAEL for convulsions (0.2 mg/kg/d)
- Martin and Hart monkey study had LOAEL for convulsions at 10 mg/kg/d with no mortality
  - Provides some confidence for safety regarding lethality using Cholakis
  - However, in Martin & Hart was small
    - Justifies additional uncertainty adjustment

Charge Question 4b. Inhalation RfC

- Neither inhalation pharmacokinetics nor inhalation toxicity studies are available. Route-to-route extrapolation of pharmacokinetics would not be supported.
  - QSAR modeling from N-nitroso compounds might be considered in the future.
- Therefore, it is reasonable not to derive an RfC.

Charge Question 4c. Oral Slope Factor for Cancer

1. The draft assessment presents an overall oral slope factor of 0.038 per mg/kg-day based on the combination of liver and lung tumors in female mice. Is this derivation scientifically supported and clearly described? NOT YET

- The OSF is dependent on needed changes for calculation of the POD for cancer as proposed by the panel in response to question 3.e.(iii).
- Conceptually the derivation of the OSF is scientifically supported, but the Panel had concerns with the clarity of the presentation.
- The idea of combining tumors from different sites is logical and toxicologically sound providing that there is biological independence. However, there is still some lack of clarity as to the design and use of the MS Combo model. EPA needs to provide a better explanation of the procedure.

- Justification was not provided for only considering multi-stage dose-response models, beyond historical precedent.
  - The near linearity of this model fit results in the relatively poor fit at the high dose levering the entire model fit toward a lower slope and, therefore, a lower POD.
  - Although the multi-stage model does ensure positive slopes throughout, the BMDS software allows other models to also adhere to this constraint.
- The Panel was also concerned that the female liver cancer concurrent controls were very low compared to available historical control rates. This low rate influences the final model for liver that in turn influences the POD and the overall OSF. This comment comes with no particular recommendation.
- Concerns that the highest dose level in the Lish et al. (1984) was above maximum tolerated dose suggest that the POD may be based on data excluding this dose level. This would change the POD which in turn changes the OSF. This comment comes with no particular recommendation.

Charge Question 4d. Inhalation Unit risk for cancer

- Available data do not support an inhalation unit risk
- There are no toxicokinetic data for inhalation of RDX
- There has not been an inhalation cancer study of RDX

Charge Question 5. Executive summary. Does the executive summary clearly and adequately present the major conclusions of the assessment?

- Generally the executive summary is well written, succinct, and clear.
- Several content and editorial comments were collated from the Panel that will be provided for EPA's consideration.
- As changes are made to the document following feedback from the Panel, the executive summary should be updated accordingly.

Suggested Edits

- Explain why the dietary exposure is “more representative of potential human exposures.”
- Too much emphasis on suppurative prostititis; the description of the urogenital effects in male rats should include specific mention of the renal effects, not only the prostatic effects.
- Simply state in a single paragraph that there is no available literature to support the identification of inhalation route hazards and reference concentration.
- Indicate some of the uncertainty or limitations in the animal cancer bioassay results.
- Add:
  - The main criteria used for choosing the principal study
  - The importance of RDX purity in published studies.
  - Concordance in doses producing convulsions and doses at which death occurred in key animal studies.
  - Summary statement addressing the confidence in the RfD